

Appelbaum/Dul 10/18/95

EXPRESSION OF TNF-LIKE PROTEIN

HGS clone 413412 = HTPAN08

Objectives:

- Express as fusion protein in *E. coli*—to be used for raising antibodies
- Express in soluble form in *E. coli* or other systems—to be used for receptor binding and activity assays

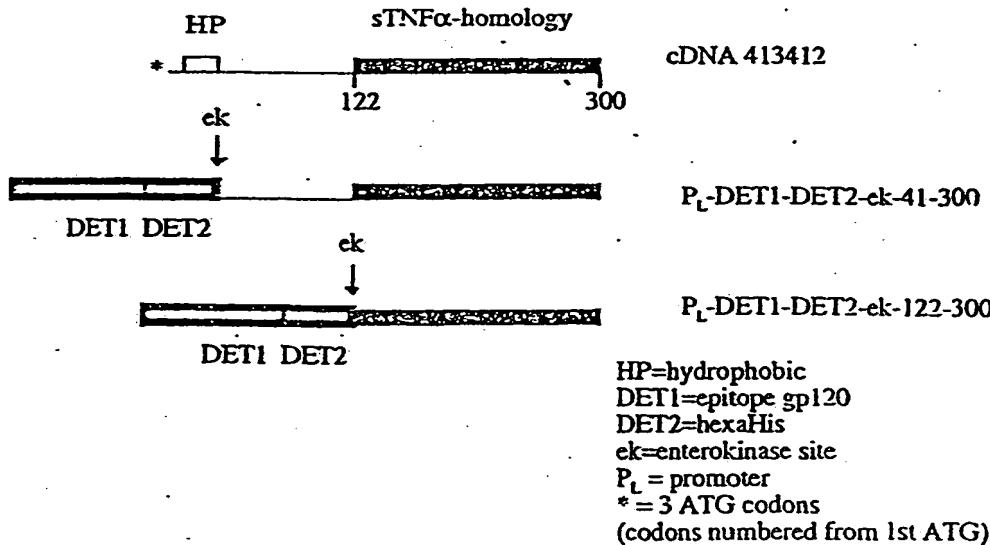
Accomplishments:

- Plasmid DNA from HGS strain (purified by K. B. Tan) was sequenced.

The DNA sequence in the open reading frame agreed with that reported by HGS.

There was a 1 bp difference upstream of the first of 3 closely spaced in-frame ATG codons, which makes it more likely that the first ATG is the translation initiator codon (TTCATGG in HGS sequence is ATCATGG in SB sequence; the latter is in good agreement with Kozak consensus upstream sequence)

- Two fusion constructs were made for *E. coli* expression; both expressed well as insoluble proteins; both have the potential for release of untagged protein following enterokinase digestion. Protein purification/rabbit injection is planned.



Ruben EXHIBIT #31

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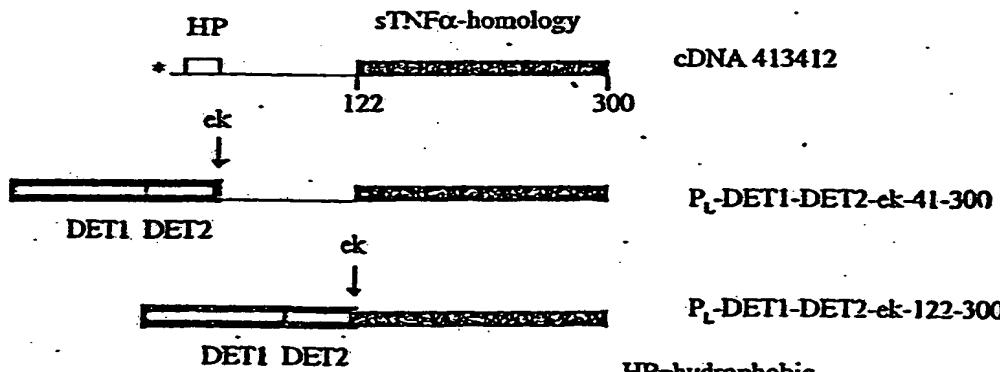
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HP=hydrophobic
 DET1=epitope gp120
 DET2=hexaHis
 ek=enterokinase site
 P_L=promoter
 * = 3 ATG codons
 (codons numbered from 1st ATG)

Ruben EXHIBIT 2031
 Ruben v. Wiley et al.
 Interference No. 105,077
 RX 2031

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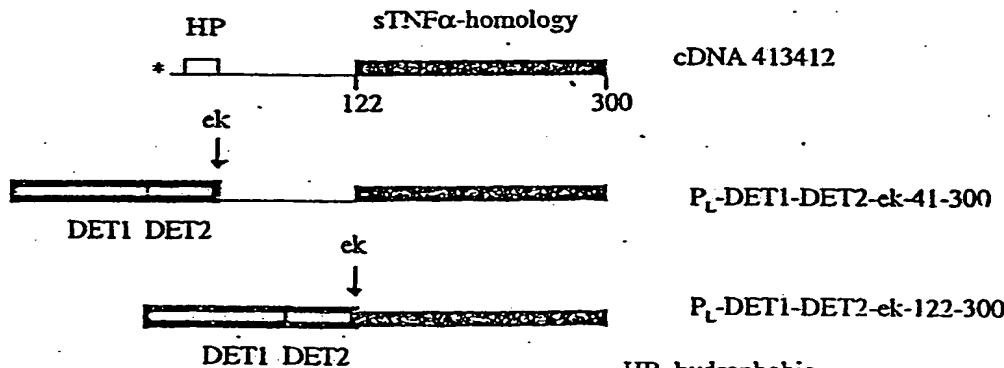
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EA1

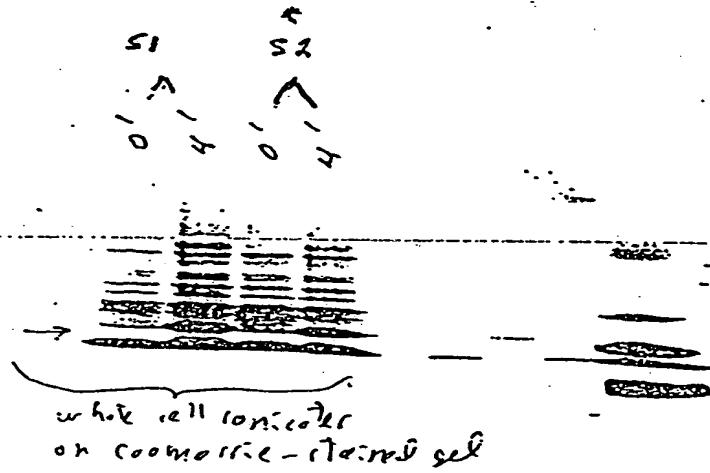
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801

Expression of clone 413412 fusions
in E. coli:

STRATAGENE EAGLEYE II 10/16/95 14:59:23

IMAGE SIZE (640 x 480 x 8).
REAL-TIME ACQUIRE.
IMAGE CREATED ON MON OCT 16 14:59:21 1995.



soluble and insoluble
fraction of cell lysates
on Coomassie-stained gel

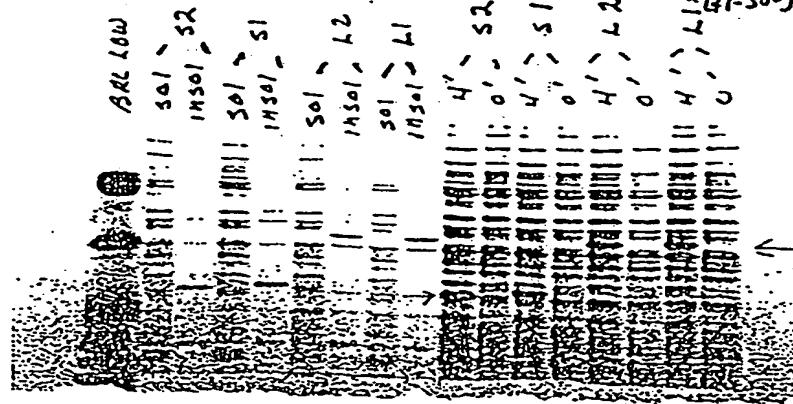
whole cell sonicates
on Coomassie-stained SDS-PAGE

STRATAGENE EAGLEYE II 10/16/95 14:53:22

IMAGE SIZE (640 x 480 x 8).
REAL-TIME ACQUIRE.
IMAGE CREATED ON MON OCT 16 14:52:19 1995.

PLA DET1
DET2
TNF-LIKE
(122-300)

PEC DET1
DET2
TNF-LIKE
(41-360)

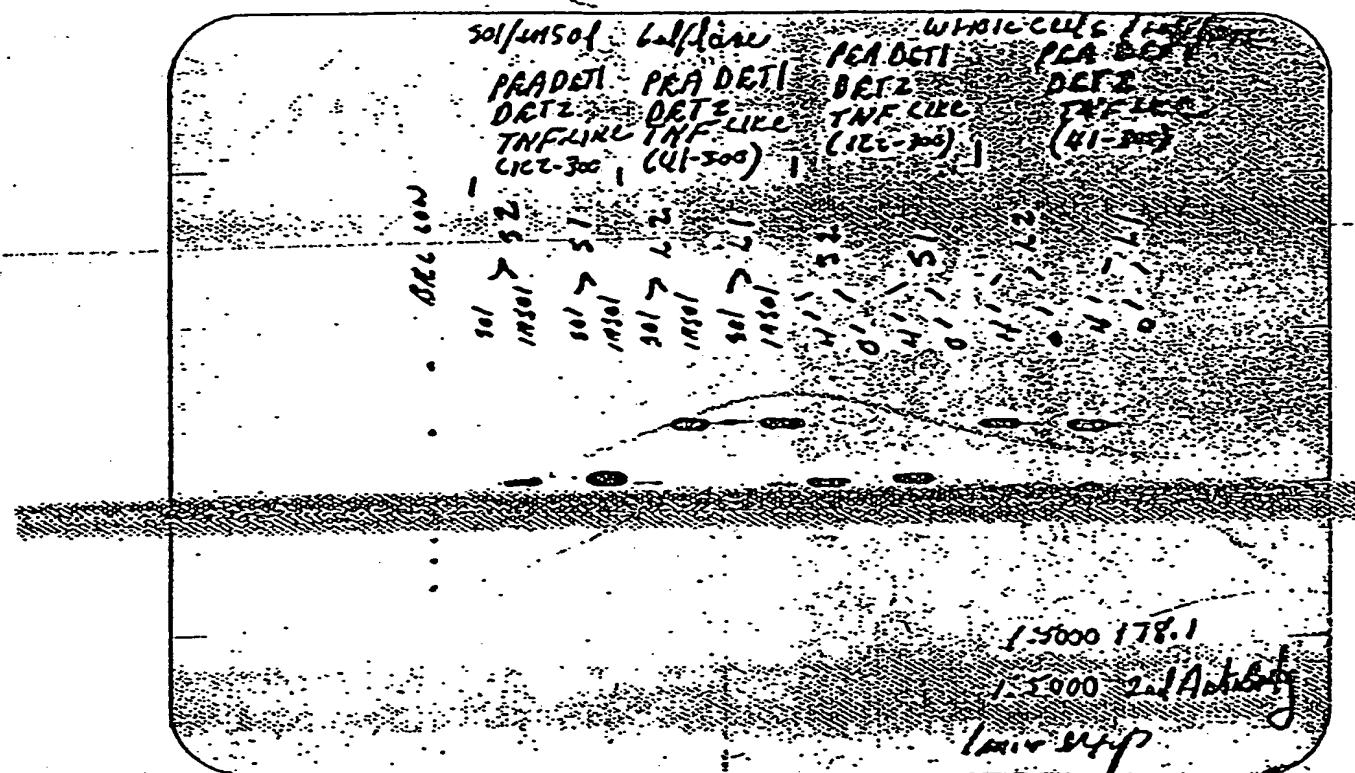


Dul

Expression of clone 413412 fusion

in E. coli -

Western blot with anti-DETF Mob



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